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Understanding Plant Responses to Drought and Salt Stresses: Advances and Challenges in “Omics” Approaches

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Abstract

Global climatic changes and the temperature-associated fluctuations in drought, soil and water salinization and flooding have resulted in huge pressure on crop plants for their optimum yield potential. These challenges have to be met through innovative scientific technologies. Recent advances in the “Omics” approaches such as transcriptomics, proteomics and metabolomics offer new dimensions for understanding plant responses to drought and salt stresses and identification of major genes/QTLs for generation of resistant germplasm. Most importantly, the proteomics coupled with bioinformatics tools have accelerated the proteins characterization at the organ, tissue, organelle and membrane levels. Here we present an update on the progress of “Omics” approaches to understand plant responses to drought and salt stress particularly in the last decade. Future challenges and solution efforts are also discussed in the ways of omics approaches. The need for research involving integrated omics technologies with advanced tools and to meet the future challenges toward practical implementation of these technologies for crop improvement against drought and salinity stresses is also discussed.

Keywords: abiotic stresses, omics, proteomics, transcriptomics, mutants, map-based cloning

1. Introduction

Abiotic stresses, particularly drought, salt and low and high temperatures adversely affect plant growth and productivity and collectively account for more than 50% yield losses in

important crop plants worldwide [1]. The resultant adverse changes in plant growth and productivity are orchestrated at the morphological, molecular and physiological levels [2]. The physiological effects of these stress conditions on plant developmental processes are mostly overlapping. Drought and salt stresses, in particular affect plants physiological and developmental processes by imposing osmotic and oxidative stresses. In addition, salt stress causes ionic stress and Na^+ toxicity. These stress conditions, in turn, induce cellular damages resulting in the disruption of ionic and osmotic [3]. In response to these stress conditions, plants generate a set of events comprising perception and transduction of stress signals. These changes ultimately result into expression of stress-related genes that induces alterations in metabolic processes [3]. The abiotic stress responses are generally polygenic in nature and are shared in multiple abiotic stresses [4].

Being a polygenic trait, achieving abiotic stress tolerance in crop plants through conventional breeding is a tedious and time-consuming approach. In this respect, comparative genomics has been utilized to explore candidate genes conferring tolerance to salt, drought and extreme temperature stresses in several plants [5, 6]. In recent years, appreciable work has been conducted to identify abiotic stress-related transcriptomes and proteomes in several plant species. The availability of these information in plants have paved the way for dissecting abiotic stress responses at the molecular level that provided a base for transgenic approaches against abiotic stresses. These approaches were utilized to engineer several crop plants in order to enhance their abiotic stress tolerance [4, 7]. However, taking into consideration the polygenic nature of abiotic stress tolerance, detailed transcriptomic and proteomic studies are required across the plant species to fully dissect the stress-response pathway. Such information will add to the current efforts to find suitable genes for plant transformation against abiotic stresses. The current review summarizes the recent findings on abiotic stress tolerance-related transcriptomic and proteomic studies in plant species.

2. Progress in functional and molecular genomics toward understanding stress perception

Abiotic stress tolerance is a polygenic trait that involves the expression of many sets of genes working in different pathways [8]. Plants have a well-organized system of sensing the environmental signals and responding to them in the form of gene expression [9]. The process of stress perception is comprised of a set of events including stress signaling, stress transduction and gene expression that result in accumulation of transcription factors, stress-related proteins, enzymes and metabolites (**Figure 1**). In order to fully understand the plants abiotic stress tolerance, and to modify it with the help of transgenic technologies, understanding the process of stress perception at the molecular level is very important. The application of functional genomics technologies has added new dimensions to our understanding of plant responses to environmental stresses [10]. The progress of abiotic stress tolerance in plants through conventional breeding programs has met with limited success, mainly because of the polygenic nature of abiotic stress responses in plants. However, during the last decade, considerable progress was made toward development of functional genomic tools that allowed the functional

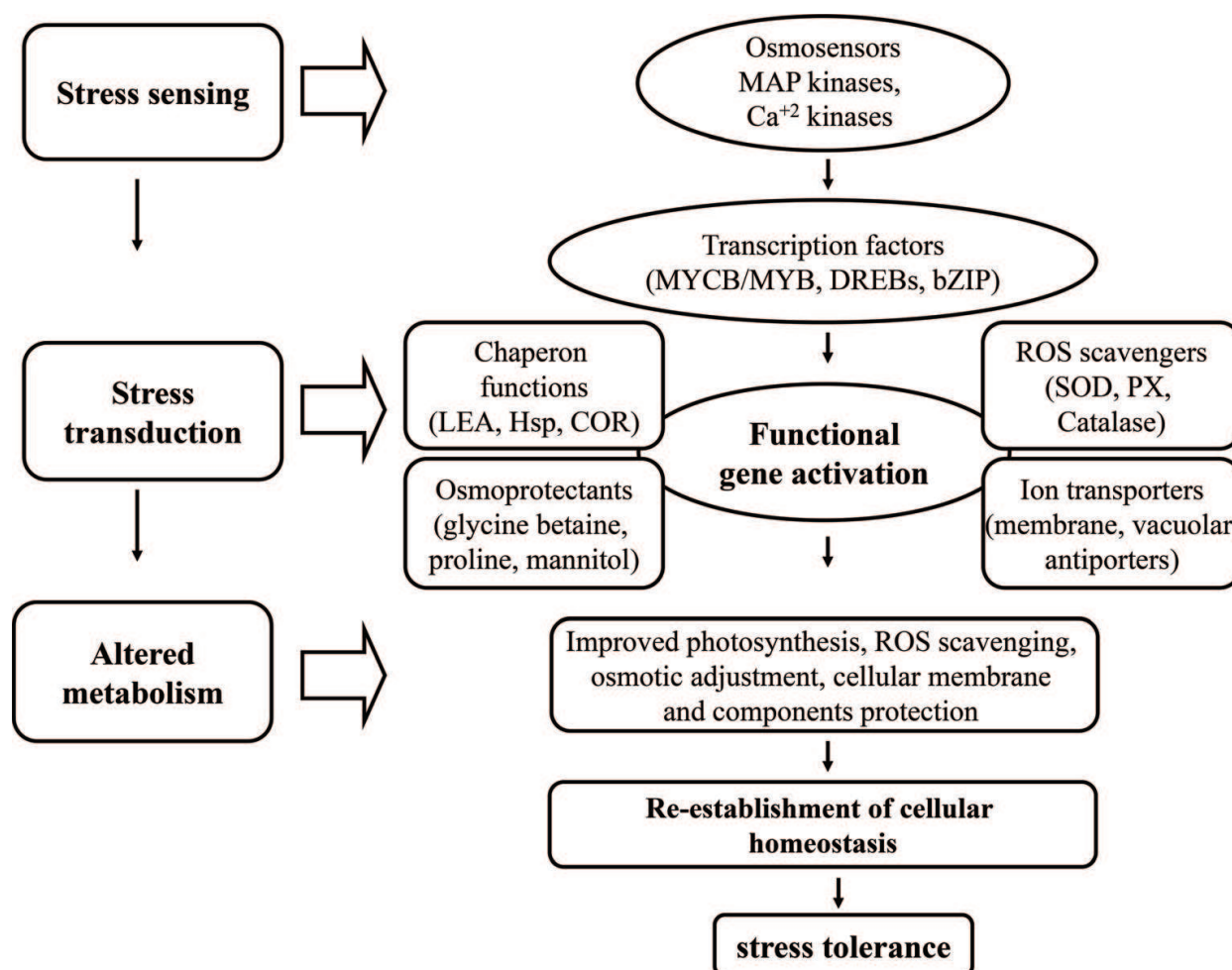


Figure 1. The process of plant response to abiotic stresses. The plant abiotic stress response pathway involves stress sensing, stress transduction and altered metabolism. Stress tolerance is achieved through expression of a large number of genes that accumulate stress-related transcription factors, chaperon function proteins, ROS scavenging enzymes, primary and secondary metabolites, osmoprotectants and cellular and vacuolar membrane antiporters.

dissection of the genetic determinants associated with abiotic stress responses. Major breakthroughs included (1) development of molecular markers for gene mapping and the construction of associated maps, (2) the development of expressed sequence tags (ESTs) libraries, (3) the complete sequencing of *Arabidopsis*, maize and rice genomes, (4) the development of T-DNA tagged mutagenic populations of *Arabidopsis* and (5) the development of forward genetics tools such as Targeting Induced Local Lesions in genomes (TILLING) technique to assess functional analysis of genes [11].

3. Map-based cloning of abiotic stress-related genes

Exploring genome sequences of *Arabidopsis* and rice and progress toward development of molecular markers and some new techniques has enabled positional cloning of mutated genes

and natural alleles. A large number of molecular markers including single nucleotide polymorphisms (SNPs), simple sequence repeats (SSRs) and insertions/deletions (InDels) are available for *Arabidopsis* and rice plants. Map-based cloning approach that uses these various molecular markers have been used to identify a large number of abiotic stress-related genes such as the salt overly sensitive (*SOS1*, *SOS2*, *SOS3*, *SOS4* and *SOS5*) genes, and other stress-responsive genes [10]. For generation of mutant lines, ethyl methane sulfonate and irradiations have been extensively used so far. In addition, the recent development of new techniques such as stress-associated genes (SAGs) and TILLING have added new dimensions in identifying mutations in stress-related genes and variant alleles [12]. In the near future, these techniques will be available for a number of crop plants such as *Arabidopsis*, wheat, maize, rice and brassica [13].

Map-based cloning strategy has also been exploited to unravel abiotic stress-related QTLs in plants. As abiotic stress tolerance trait is polygenic in nature, the QTLs studies have received immense importance in understanding stress responses [14]. Recently, using map-based cloning, a large number of drought and salt stress-related QTLs have been reported in crop plants. QTLs were mapped in *Oryza sativa* for abiotic stress tolerance [15, 16], *Brassica napus* for salt tolerance [17], maize for salt tolerance [18], wheat for drought tolerance [19] and cotton for salt tolerance [20]. Gene stacking approach through marker-assisted selection was successfully used in an elite rice cultivar for stacked QTLs related to biotic and abiotic stresses (submergence and salinity tolerance) [21, 22]. Two out of 10 pyramid lines showed adequate tolerance to all tested stresses including abiotic stresses. Similar studies using abiotic stress tolerance genes/QTLs need to be extended to other crop plants.

4. Development of mutant populations

The use of mutant populations of plants, developed through insertional mutagenesis is an important tool to dissect the functions of abiotic stress-related genes [23]. Insertional mutagenesis is accomplished through T-DNA or transposable elements. Such mutant populations are available for *Arabidopsis* and rice plants. These saturation mutant populations of *Arabidopsis* and rice cover more than 90% of their genes that could be employed for characterization of abiotic stress tolerance genes [24]. Development of high throughput genomic platforms such as serial analysis of gene expression (SAGE), HRM (differential display, high resolution melt) analysis, TILLING, microarray, etc. have made rapid analysis of these mutation events. A large number of abiotic stress-related genes have been identified using *Arabidopsis* and rice knockout populations. In a 250,000 independent T-DNA insertional *Arabidopsis* population, more than 200 mutants were found with altered stress responses. Some of these include mutations in genes encoding transcription factors, ABA biosynthetic enzymes and sodium transporter high affinity K⁺ transporter (HKT1) [25]. Recent progress on the generation of T-DNA insertion lines have been reviewed in several articles [26, 27].

Along with T-DNA and transposable elements based mutant populations; the need for alternative means of studying gene function is growing day by day. This is mainly because of the

low number of *Arabidopsis* and rice tagged genes that code for clear phenotypes [28]. Recently, traps and activation tagging have been focused as the alternative means of gene tagging [29, 30]. Trap and activation techniques have been widely used for generation of tagged populations of *Arabidopsis* and rice.

5. Transcriptomic analysis

Progress in transcriptomic analysis tools has revealed massive genomic sequence information in many plants. Identification of the partial or complete cDNAs sequences provide a holistic picture of the transcriptomes. The available ESTs are organized in three main databases, that is, NCBI, TIGR and Sputnik, which organize these ESTs with fully characterized gene sequences. Abiotic stress-related ESTs have contributed a great deal in exploring gene expression profiles of stress tolerance-related traits in *Arabidopsis* and rice [31].

In recent years, different functional and molecular tools were used to identify abiotic stress-responsive genes in plants. These included genome wide physical and genetic mapping of chromosomes, isolation and sequencing of genes, ESTs, proteomics techniques and cDNA microarray analysis [32]. Particularly, the cDNA and microarrays were widely used to study gene expression profiles in *Arabidopsis*, potato, rice, sorghum, maize and wheat under abiotic stresses. The identified genes/proteins include late embryogenesis abundance (LEA) proteins, compatible osmolytes, ROS scavengers and proteins involved in signal transduction.

The genomic approaches related to abiotic stress tolerance in plants are summarized (**Table 1**). In one study, Oono et al. [33] used a full-length cDNA microarray containing 7000 *Arabidopsis* full-length cDNAs and identified 152 rehydration-inducible genes. Among the 152 rehydration-inducible genes, 58 genes showed proline- and hypoosmolarity-inducible gene expression. Similar study was conducted in *Arabidopsis* under drought stress [34]. Transcriptomic analysis of *M. sativa* and *M. esculenta* revealed expression of several genes responsive to salt and drought, respectively [35, 36]. In rice plants, the pioneering work came from Rabbani et al. [37]. They used cDNA and gel microarray analysis to identify cold, drought, salinity and ABA inducible genes. They identified 73 stress inducible genes, among which 15 genes were highly responsive to all four treatments. Lan et al. [38] determined and compared the drought and wounding stress-related gene expression profiles. Drought stress regulated many of the pollination/fertilization-related genes. Similarly, the drought stress-related transcriptomic analysis was conducted in some other studies in rice [39]. Using a cDNA microarray, 486 salt responsive ESTs were determined in shoots of rice plants under salt stress [40]. Moreover, Hmida-Sayari et al. [41] used the cDNA amplified fragment length polymorphism (AFLP) technique to investigate the expression profile of potato under salt stress. The expression profile showed 5000 bands, of which 154 were up-regulated, while 120 were down-regulated. Most of these ESTs were found to have a role in biotic and abiotic stresses. Sequence comparison of some of these fragments revealed close homologies with proteins, involved in cell wall structure, stress proteins such as glyceraldehyde dehydrogenase and proteins related to hypersensitive response to pathogens. Approximately 20,000 ESTs were generated from a cDNA library constructed

Species	Stress type	Findings	Reference
<i>Arabidopsis thaliana</i>	Drought	Total of 152 rehydration-inducible genes were identified.	Oono et al. [33]
<i>A. thaliana</i>	Drought	Translational regulation of 2000 genes was evaluated	Kawaguchi et al. [34]
<i>Medicago sativa</i>	Salt	Expression of large number of genes including 86 transcription factors was altered significantly	Postnikova et al. [35]
<i>Manihot esculenta</i>	Drought	Up-regulation of 1300 drought-responsive genes	Utsumi et al. [36]
<i>Oryza sativa</i>	Salt, drought	73 stress inducible genes were identified, among which 15 genes were highly responsive to salt, drought and cold stresses	Rabbani et al. [37]
<i>Oryza sativa</i>	Drought	53.8% and 21% of the pollination/fertilization-related genes were regulated by dehydration and wounding, respectively	Lan et al. [38]
<i>Oryza sativa</i>	Drought	—	—
<i>Oryza sativa</i>	Drought	589 genes were found responsive to drought	Gorantla et al. [14]
<i>Oryza sativa</i>	Drought	About 55% of genes differentially expressed in roots of rice under drought stress	Moumeni et al. [39]
<i>Oryza sativa</i>	Salt	486 salt responsive ESTs were determined in shoots	Chao et al. [40]
<i>Oryza sativa</i>	Drought, salt	Differential expression of large number of genes encoding transcription factors in stress sensitive and tolerant genotypes	Shankar et al. [47]
<i>Solanum tuberosum</i>	Salt	Six ADP-ribosylation factors like proteins were identified.	Kim et al. [110]
<i>Solanum tuberosum</i>	Salt	Expression profile showed 5000 ESTs, of which 154 were up-regulated, and 120 were down-regulated	Hmida-Sayari et al. [41]
<i>Solanum tuberosum</i>	Salt, heat, drought	1476 stress-related ESTs were found	Rensink et al. [42]
<i>Solanum tuberosum</i>	Salt, heat	3314 clones were identified as up- or down regulated	Rensink et al. [43]
<i>Sorghum bicolor</i>	Drought	333 genes responded to ABA, NaCl or osmotic stress	—
<i>S. bicolor</i>	Drought	775 genes were found differentially expressed in response to drought stress	Pratt et al. [44]
<i>S. bicolor</i>	Drought	Differential expression of genes involved in photosynthesis, carbon fixation, antioxidants in sensitive and tolerant genotypes	Fracasso et al. [49]
<i>Triticum aestivum</i>	Salt	Gene expression of 1811 genes was changed in response to salt stress	—
<i>Triticum aestivum</i>	Drought	3831 transcripts showed changes in expression in the drought-tolerant genotype	Li et al. [45]
<i>Triticum aestivum</i>	Drought	Large number of genes including 309 differentially expressed genes, responsive to drought stress were up-regulated	Ma et al. [48]
<i>Zea mays</i>	Water stress	79 genes in placenta and 56 genes in endosperm, were up- and down regulated, simultaneously	—

Species	Stress type	Findings	Reference
<i>Zea mays</i>	Drought	Differential expression levels of cell-wall related and transporter genes were found to contribute to drought tolerance	Zheng et al. [46]
<i>Zea mays</i>	Drought	A total of 619 genes and 126 transcripts were identified whose expression was altered by drought stress	Song et al. [50]

Table 1. Drought and salinity stress-responsive transcriptomic studies in various plant species.

from potato leaves and roots, which were subjected to salt, heat, cold and drought stresses [42, 43]. Some of these ESTs were found to have sequence similarities with abiotic stress-responsive genes in other plant species. Similar transcriptomic studies were conducted in some other plants such as sorghum [44], wheat [45], and maize [46] subjected to drought and salt stresses.

Recently, transcriptomic analysis through RNA sequencing has been proved to be a powerful tool for analysis of drought and salt stress-responsive genes. RNA-Seq uses next generation sequencing to reveal quantities of RNA in a given sample in real time. Examples of transcriptomic analysis through RNA-Seq have been reported in several crop plants subjected to drought and salt stresses. Shankar et al. [47] studied comparative transcriptomic analysis in drought sensitive and tolerant rice cultivars. A total of 801 and 507 transcripts were found differentially expressed in drought-tolerant (N22) and salt-tolerant (Pokkali) rice cultivars, respectively, under stress conditions. Overall, the study identified common and cultivar-specific stress-responsive transcripts. Ma et al. [48] conducted RNA-Seq analysis in wheat to study the drought-responsive transcriptomic changes during reproductive stages under field conditions. A total of 115,656 genes were detected and among these, 309 genes were found differentially expressed under drought at various developmental stages. Fracasso et al. [49] conducted transcriptomic analysis to study responses of drought sensitive and tolerant sorghum genotypes subjected to drought stress. Several genes such as those involved in photosynthesis, carbon fixation and antioxidants were found differentially expressed in the two genotypes under drought stress. Correlation in maize flowering time and drought stress was studied through RNA-seq and bioinformatics tools [50]. A total of 619 genes were identified, among which the expression of 126 transcripts was altered by drought stress. Among drought-responsive genes, the important transcripts included zinc finger and NAC domains. The study also identified 20 genes such as transcription factor *HY5*, *PRR37* and *CONSTANS* involved in flowering times.

The above-mentioned transcriptomic studies revealed that RNA-Seq analysis could be used as a very powerful tool not only to study stress-specific gene expression analysis but also to explore differences between stress sensitive and tolerant genotypes of crop plants.

6. Proteomic analysis

The study and characterization of the complete set of proteins in a cell, organ or organism at a given time is termed as proteomics [51]. Along transcriptomic studies, proteome analysis has

contributed much to our understanding of the expression of stress-related genes in plants under abiotic stress. Proteomic studies on plant responses to salinity and drought stresses are being explored at large scale. Proteomic approaches have been applied at whole plant, organ and at subcellular levels to unravel the stress-response mechanism in plants. The prominent proteomic studies in plant species facing drought and salinity stresses are summarized (Table 2). Proteomic studies on sugar beet under drought stress identified that heat-shock proteins, nucleoside diphosphate kinase, RuBisCO, Cu-Zn superoxide dismutase (SOD) and 2-Cys-peroxiredoxin were highly induced [52]. Kim et al. [53] conducted proteomic analysis of maize subjected to drought stress and identified proteins involved in metabolism, photosynthesis and stress responses. Proteomic analysis of *Arabidopsis* under drought stress revealed that branched-chain amino acid amino transferase 3 protein and zinc finger transcription factor oxidative stress 2 proteins had a significant role in drought stress responses in the plants that over-expressed ethylene response factor AtERF019 [54].

Species	Stress	Proteomic changes	Plant organ/ organelle	Reference
<i>Beta Vulgaris</i>	Drought	79 proteins showed significant changes under drought. Important were RuBisCO and 11 others involved in redox regulation, oxidative stress, signal transduction and chaperone activities	Leaf	Hajheidari et al. [52]
<i>Oryza sativa</i>	Drought	Out of 12 proteins, 10 were up-regulated and 2 were down-regulated. These were mainly grouped as defense, energy, metabolism, cell structure and signal transduction proteins	Leaf sheath	Ali and Komatsu [116]
<i>Triticum durum</i>	Drought	Out of 36 significantly changed proteins, 12 were increased in abundance while 24 were decreased. RuBisCO large subunit, triose phosphate isomerase, thiol-specific antioxidant protein, phosphoglycerate kinase were increased	Leaf	Caruso et al. [58]
<i>Helianthus annuus</i>	Drought	Six proteins related to stress and carbon metabolism were found significantly up-regulated in leaves of drought stressed sunflower leaves.	Leaf	—
<i>Glycine max</i>	Drought	32 proteins changed in root. HSP 70, actin B and methionine synthase were differentially changed in the 3 organs	Root Hypocotyl Leaf	Mohammadi et al. [59]
<i>Brassica napus</i>	Drought	35 proteins in sensitive and 32 in tolerant line were differentially expressed. Six proteins in F1 hybrid were common to sensitive and tolerant lines	Root	Mohammadi et al. [60]
<i>Oryza sativa</i>	Drought	Out of 900 identified proteins, 38% were changed in abundance compared to non-treated. Pathogenesis-related, chitinases and redox proteins were increased while tubulins and transport-related proteins were decreased.	Root	Mirzaei et al. [61]
<i>Vitis vinifera</i>	Drought	Early responding proteins included photosynthesis, glycolysis, translation, antioxidant defense, while late-responding proteins included transport, photorespiration, antioxidants, amino acid and carbohydrate metabolism	Shoot	Cramer et al. [117]

Species	Stress	Proteomic changes	Plant organ/ organelle	Reference
<i>Zea mays</i>	Drought	Identified proteins were involved metabolism, stress response, photosynthesis, and protein modification	Leaves	Kim et al. [15]
<i>Glycine max</i>	Drought	643 proteins were significantly changed in soybean seedlings recovering from drought stress. Majority of these proteins belonged to stress, hormone metabolism, glycolysis and redox categories.	Root including hypocotyl	Khan and Komatsu [64]
<i>Zea mays</i>	Drought	Abundance of 68 proteins was changed. Out of these, 46 proteins were increased while 22 were decreased. Asparagine synthetase, alpha-galactosidase, fatty acid desaturase and plastid proteins were among the highly changed proteins	Leaf	Zhao et al. [118]
<i>Brassica napus</i>	Drought	Abundance of 138 proteins was differentially changed. Drought-responsive differentially abundant proteins were involved in signal transduction, photosynthesis and glutathione-ascorbate metabolism.	Leaf	Wang et al. [67]
<i>Solanum lycopersicum</i>	Drought	A total of 31 proteins were differentially changed in abundance under drought and 54 were changed during recovery phase. ABA accumulation pointed activation of chloroplast to nucleus signaling pathway	Leaf	Tamburino et al. [65]
<i>Phaseolus vulgaris</i>	Drought	Abundance of HSP-70 protein was highly changed. Protein synthesis, proteolysis and folding-related proteins increased in abundance	Stem	Zadraznik et al. [66]
<i>Brassica napus</i>	Drought	Among the 79 significant identified proteins, nitrogen assimilation, and ATP and redox Homeostasis were up-regulated in water savers cultivars; while photosynthesis, carbohydrate, RNA processing and stress related proteins were increased in water spender cultivars during water stress	Leaf	Urban et al. [68]
<i>Glycine max</i>	Salt	Under 100 mM salt stress, seven proteins were found to be up- or down-regulated. LEA, b-conglycinin, elicitor peptide three precursor, and basic/helix-loop-helix protein were up-regulated. While protease inhibitor, lectin, and stem 31-kDa glycoprotein precursor were down-regulated	Root Hypocotyl	Aghaei et al. [71]
<i>Hordeum vulgare</i>	Salt	ROS scavenging proteins were up-regulated in the tolerant genotype, while iron uptake proteins were up-regulated in the sensitive one	Root	Witzel et al. [73]
<i>Nicotiana tabaccum</i>	Salt	Total 18 proteins were differentially expressed under salt stress. Photosynthesis related proteins were up-regulated while defense-related proteins were down-regulated	Leaves	—
<i>Solanum lycopersicum</i>	Salt	Total 23 salt stress-responsive proteins belonging to six functional groups were identified	Root, Hypocotyl	Chen et al. [119]
<i>Glycine max</i>	Salt	Metabolism-related proteins were found up- and down-regulated in leaves, hypocotyls and roots under salt stress	Root, Hypocotyl	Sobhanian et al. [75]

Species	Stress	Proteomic changes	Plant organ/ organelle	Reference
<i>Phoenix dactylifera</i>	Salt, drought	The levels of ATP synthase alpha and beta subunits, RuBisCO, photosynthesis and ROS-related proteins were significantly changed under both stresses	Leaves	El Rabey et al. [120]
<i>Triticum aestivum</i>	Salt, Drought	Of the total 124 stress responsive proteins, 26.61% were induced by drought, included chaperonin, cys-peroxiredoxin, ethylene response, and elongation factor; while 23.38% were induced by salinity stress, included bowman-birk type protease inhibitor, calcineurin B-like protein, cyclophilin and RNA binding proteins	Seed	Kamal et al. [121]
<i>Oryza sativa</i>	Salt	In the two different cultivars, 104 and 102 proteins were significantly altered. Actin-7, tubulin alpha, V-type proton ATPase, SOD and pyruvate decarboxylase were among the observed salt-induced proteins	Root	Damaris et al. [80]
<i>Avena sativa</i>	Salt	From 30 differential protein spots, protein related to calvin cycle, adenosine-triphosphate regulation-related and 50S ribosomal proteins decreased while antioxidant enzymes abundance were increased.	Leaf	Bai et al. [78]
<i>Triticum aestivum</i>	Salt	Out of total of 121 proteins, ubiquitination-related proteins, transcription factors, pathogen-related proteins and anti-oxidant enzymes were increased for homeostasis	Root	Jiang et al. [122]

Table 2. Drought and salinity stress-related proteomic studies in various plant species.

In addition to the above-mentioned studies of proteomic analysis on the whole plant level, some notable studies have also focused the impact of drought and salinity stresses on organ-specific proteomic constituents. The metabolism-related proteins such as the isoflavone reductase, were observed as down-regulated which possibly played an important role in plant defense against various stresses [55]. Leaf-specific protein analysis in other plants identified drought-responsive proteins. These studies were conducted in rice [56], sunflower [57], wheat [58] and soybean [59, 60]. Root-specific proteome analysis was conducted in a number of crops under various drought stress, which identified a wide range of proteins including those involved in pathogenesis, transport and oxidation-reduction reactions. Prominent studies were conducted in canola (*Brassica napus*) [60], soybean [59] and rice [61]. Similar studies were conducted in rice [62] and wheat [63] subjected to salt stress, which identified changes more prominently in metabolism-related gene expression. Khan and Komatsu [64] performed proteomic analysis of soybean root including hypocotyl during recovery from drought stress and concluded that peroxidase and aldehyde dehydrogenase scavenge toxic reactive oxygen species and reduce the load of harmful aldehydes for helping the plant to recover. In tomato facing drought stress, chloroplast to nucleus signaling pathway in connection to abscisic acid (ABA) signaling network was activated [65]. In common bean stem, heat-shock protein 70 was highly increased in abundance suggesting its role in restoration of normal conformations of proteins for cellular homeostasis [66]. Proteomic analysis of maize leaves under drought stress revealed that ABA regulates the signaling pathways pertaining to oxidative phosphorylation,

photosynthesis and glutathione metabolism. Phosphorylation of β carbonic anhydrase 1 imparted adaptation to drought stress in *Brassica napus* [67]. Proteomic analysis of rapeseeds under drought stress indicated that nitrogen assimilation, oxidative phosphorylation, redox homeostasis, energy, photosynthesis and stress-related proteins were raised in abundance in different cultivars [68].

Salinization of arable lands may result in up to 50% land loss by the year 2050 [69]. Proteomic techniques have been employed for analyzing salt stress responses in plants. In salt-tolerant and -sensitive potato cultivars, photosynthesis-related proteins were down-regulated; whereas osmotin-like proteins, heat-shock proteins and protein inhibitors were up-regulated [70, 71]. In soybean, β -conglycinin, elicitor peptide three precursor, late embryogenesis-abundant protein, and basic/helix-loop-helix protein, were up-regulated, suggesting soybean adaptation to salt stress; whereas protease inhibitor, lectin and stem, 31-kDa glycoprotein precursor were down-regulated, suggesting the weakening of plant defense system under the salinity stress [72]. Differentiation of salt stress-related proteins was evaluated in tolerant and sensitive barley genotypes [73]. Another study conducted on barley found expression of germin-like and pathogenesis-related proteins important for salt stress responses [74]. ATP production-related glyceraldehyde-3-phosphate was down-regulated in soybean under salt stress [75]. Cupin domain protein 3.1 was revealed in enhancing seed germination in rice under salt stress [76]. In barley, salt stress increased the abundance of proteins related to anti-oxidation, signal transduction, protein biosynthesis, ATP generation and photosynthesis [77]. Proteomic analysis of oat leaves under salt stress indicated decrease in abundance of calvin cycle-related and adenosine-triphosphate regulation-related proteins; whereas antioxidant enzymes level was increased [78]. Alterations in proteomic profiles were recorded in wheat cultivars under salt stress [63]. Kamal et al. [79] reported a decrease in ATP synthase and V-type proton ATPase subunits; whereas cytochrome b6-f, germin-like-protein, glutamine synthetase, fructose-bisphosphatealdolase, S-adenosylmethionine synthase and carbonic anhydrase were gradually increased. Damaris et al. [80] reported induction of actin-7, tubulin alpha, V-type proton ATPase, SOD and pyruvate decarboxylase in salt-stressed wheat cultivars. Proteomic analysis of wheat roots indicated differential expression of a number of proteins such as transcription factors, proteins related to ubiquitination pathogenesis and antioxidant enzymes under salt stress [81]. All the above discussed studies show the importance of proteomics in unraveling the vital information about the plants responses to abiotic stresses such as drought and salinity stress responses.

7. Metabolomic analysis

Metabolomics is one of the most important “Omics” technologies that can be applied to different organisms with little or no modification. The term metabolomics was introduced by Nicholson et al. [82], and since then it has been utilized extensively in agricultural research [83, 84]. The metabolite profiling provides valuable information on the stress tolerance mechanisms and may be applied to bioengineer plants with improved stress tolerance. Metabolomics studies reveal information about compounds involved in acclimation to the stress, those which

are by-products as a result of disruption of normal homeostasis and those involved in signal transduction in response to the stresses [85]. Due to involvement of metabolites in important life processes, the field of metabolic profiling could contribute significantly to the study of stress biology in plants. Both primary and secondary metabolites have been shown to play important roles in responses of plants to drought and salinity stresses. Primary metabolites such as sugars, amino acids and intermediates of Krebs cycle were found with important roles in photosynthetic dysfunction and osmotic readjustment. While, the secondary metabolites such as antioxidant scavengers, coenzymes and regulatory molecules responded to specific stress conditions. Both qualitative and quantitative studies of metabolites in response to abiotic stress are helpful in not only determining the phenotypic response of the plant and screening for stress tolerant lines but also reveal the genetic and biochemical mechanisms underlying the stress condition [86].

Drought and salt stresses affect the process of photosynthesis, affecting CO₂ diffusion leading to photorespiration and hydrogen peroxide production, causing cell damage [87]. Most recently, Rabara et al. [88] analyzed the metabolomics profile of tobacco and soybean roots and leaves facing dehydration stress. The study revealed highest tissue specific accumulation of 4-hydroxy-2-oxoglutaric acid in tobacco roots and coumestrol in soybean roots; indicating 4-hydroxy-2-oxoglutaric acid and coumestrol can be used as markers for drought stress. Metabolomic analysis of intense drought-stressed grapevine leaves was conducted to reveal induction of several metabolites [89]. Metabolomic profiling of *Arabidopsis* exposed to drought and heat stresses in combination revealed accumulation of sucrose, maltose and glucose [90]. In tolerant and sensitive thyme facing water stress, metabolomics analysis revealed differential changes in carbohydrates, amino acids, fatty acids and organic acids profiles [91]. Metabolites related to the mechanisms of osmotic adjustment, ROS scavenging, cellular components protection and membrane lipid showed significant changes. Metabolomic and proteomic analysis of xylem sap in maize under drought stress revealed a higher abundance of cationic peroxidases, which with the increase in phenylpropanoids may lead to a reduction in lignin biosynthesis in the xylem vessels and could induce cell wall stiffening [92]. Catola et al. [93] reported that trans-2-hexenal showed a significant increase in water-stressed and recovered leaves respect to the well-watered ones in pomegranate plants. This indicated a possible role of the oxylipin pathway in the response to water stress. Metabolites changes in rice grains during water-stressed and recovery indicated involvement in stress signaling pathways such as gamma-amino butyric acid (GABA) biosynthesis, sucrose metabolism and antioxidant defense [94]. Zhang et al. [95] reported that myo-inositol and proline had striking regulatory profiles in *Medicago* indicating involvement in drought tolerance. Metabolite profiling of hybrid poplar genotypes revealed that amino acids, the antioxidant phenolic compounds catechin and kaempferol, as well as the osmolytes raffinose and galactinol exhibited increased abundance under drought stress, whereas metabolites involved in photorespiration, redox regulation and carbon fixation showed decreased abundance under drought stress [96]. Concentrations of flavonoids, glycosides of kaempferol, quercetin and cyanidin were found in *Arabidopsis* during drought stress [97].

Salinity stress has been investigated at metabolite level to reveal the response mechanism. In salinity-stressed barley plants, cell division and root elongation was found associated with accumulation of amino acids, sugars and organic acids [98]. Chen and Hoehenwarter [99] reported that sucrose, fructose, glycolysis intermediates and amino acids levels were altered

in *Arabidopsis* under salinity stress. Further, metabolite changes were found positively correlated with growth potential and salt tolerance in rice genotypes for allantoin and glutamine [100]. Meulebroek et al. [101] carried out metabolomic profiling of tomato carotenoid content under salt stress. The results revealed that metabolites had several roles at the fruit level in salinity response; however, 46 metabolites had ascribed a noticeable role in carotenoid metabolism as well. In barley, concentrations of most amino acids such as 4-hydroxy-proline, arginine, citrulline, glutamine, phenylalanine, proline and amines increased significantly in roots facing salinity stress [102]. Behr et al. [103] carried out metabolomics analysis in *Suaeda maritima* exposed to salinity stress. Results revealed increase in metabolites associated with osmotic stress and photorespiration; furthermore, alanine fermentation was enhanced. Oxidative stress produced by salinity in roots of *Salicornia herbacea* induced defense metabolites such as shikimic acid, vitamin K1 and indole-3-carboxylic acid that are generated as a result of defense mechanisms, to protect against ROS [104]. Metabolomic profiling studies revealed that sugars, sugar alcohols, proline, TCA cycle intermediates, histidine, glutathione and GABA were accumulated in *Arabidopsis thaliana* under salt stress [105, 106]. Production of signaling molecules such as serotonin and gentisic acid increased in salt-tolerant varieties indicating their importance as biomarker. Ferulic acid and vanillic acid were also produced in high levels. In the salt sensitive varieties, elevated levels of 4-hydroxycinnamic acid and 4-hydroxybenzoic acid were found in the leaves [19]. Epidermal bladder cells help in salt dumping, improved potassium retention in leaf mesophyll and space provision for storage of metabolites [107]. The above discussion revealed that metabolomics is very important tool in investigating abiotic stress-response mechanisms such as those observed in drought and salt stresses.

8. The way forward

RNA-Seq and genome sequencing and proteomic techniques/technologies (2D, iTRAQ, MALDI, gel-free, label-free, LC-MS/MS-based technologies) have widened the dimensions of analyzing plant responses to abiotic stresses such as drought and salinity. Recent advances in the omics technologies have contributed considerably to our understanding of the plant abiotic stress-responsive mechanisms. In addition to advancing research in other related areas, emphasis has been on the proteomic analysis specific to whole plants, individual organs, tissues and cells [55]. These technologies are helping to characterize individual proteins specific to different organs, tissues and cells subjected to various abiotic stresses. Advanced proteomic information, coupled with other omics approaches would further strengthen the efforts to develop breeding programs based on identification of novel proteins/genes and their integration through marker-assisted selection. However, further efforts are required to focus on individual target points associated with “Omics” technologies and their application to dissect stress-responsive mechanisms. Research needs to be focused on several fronts such as more studies that target post translational modifications (PTMs), cell type-specific proteome analysis, advanced mapping populations in crop plants and comparative proteomic studies. PTMs of proteins may change their stability, subcellular localization, interactions with other proteins and ultimately proteins functioning. A number of studies revealed the important role of PTMs in protein functioning. Studies have been conducted to analyses protein phosphorylation in maize

[108, 109], phosphorylation and ubiquitination in *Arabidopsis* [110, 111] and glycosylation in soybean [112] under various abiotic stresses. In addition to improved methodologies, identification of more PTMs would unravel functional characterization of important proteins involved in stress-responsive mechanisms and plant adaptation to various abiotic stresses.

Individual proteins characterization and quantification is essential to fully explore the stress-responsive mechanisms in organs, tissues and cells. However, problems may arise due to the conventional methodologies such as protein detection on 2-DE gels [55]. Improved extraction methodologies may overcome such problems. Poor proteome coverage may be the result while detecting leaf proteome with abundance of RuBisCO that constitutes almost half of the total leaf proteins. However, proteome coverage may be improved with the recently adopted fractionation of crude protein extract. Similarly, quantification of stress responsive low abundance target proteins may be improved through selected reaction monitoring (SRM) technique [113, 114]. Such improved techniques would also help unravel commonly expressed proteins in different organs under multiple abiotic stresses. These advanced techniques coupled with improved bioinformatics approaches may help shed further light on plant responses to abiotic stresses. Recently, transgenic plants conferring abiotic stress tolerance have entered vigorous evaluations under greenhouse and field conditions. Comparative proteomic studies of these transgenic plants may be helpful to characterize key stress-responsive factors among large number of commonly expressed proteins. Identification of major stress-responsive proteins coupled with advances in transcriptomics, metabolomics and bioinformatics tools would help unravel the complex interactions among stress-responsive signaling pathways. Moreover, omics approaches such as proteomics can be extremely helpful in analyzing post-stress recovery responses in the plants, revealing the key proteins/genes involved in the recovery stage [115].

9. Conclusions

Different omics tools have been exploited to unravel plant responses to drought and salt stresses. However, further studies should be conducted to integrate multiple omics approaches including phenomics coupled with RNA-Seq and state-of-the-art proteomic technologies. These future developments will provide further impetus to the ongoing efforts of developing drought- and salt-tolerant plants with comparatively improved growth and yield potential under realistic field conditions.

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